

# Molecular typing of *Mycobacterium tuberculosis* isolates from Qinghai province of northwest china by spoligotyping and 15-locus MIRU-VNTR

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Li B, Wang Z, Ma Y et al. Molecular Typing of *Mycobacterium tuberculosis* Isolates from Qinghai Province of Northwest China by Spoligotyping and 15-Locus MIRU-VNTR. *J Microbiol Biotechnol Rep*. 2018;2(1):1-4.

Tuberculosis (TB) is a major public health concern in Qinghai which located in the northeastern region of Qinghai-Tibet Plateau and characterized by low oxygen and low pressure. This study was conducted to explore the genotypes of *Mycobacterium tuberculosis* (MTB) in this high altitude region. A total of 251 MTB strains were collected and genotyped by spoligotyping and 15-loci mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) typing. Spoligotyping of 251 MTB isolates revealed 49 distinct spoligotype patterns and Beijing lineage was found to be the

most dominant MTB strain including 74.0% (185/251) of total isolates. 217 isolates grouped into 15 clusters with the clustering rate of 86.5% (217/251). While 15-loci MIRU-VNTR distributed 251 isolates into 4 groups containing 238 different MIRU-VNTR profiles, Group II was the largest group representing 91.9% (230/251) of isolates. Clustering rate by MIRU-VNTR was 85.7% (215/251). Spoligotyping and MIRU-VNTR matched well in lineage classification. We concluded that 15-loci MIRU-VNTR has more abundant diversity than spoligotyping and would be useful to trace transmission routes and sources of infections.

**Key Words:** Qinghai-Tibet Plateau; *Mycobacterium tuberculosis* (MTB); Spoligotyping

## INTRODUCTION

Tuberculosis (TB) is a common contagious disease of human beings, caused by bacillus *Mycobacterium tuberculosis* (MTB), which caused an estimated 10.4 million new TB cases and 1.8 million deaths worldwide in 2015. Most of the estimated number of cases occurred in developing countries, mainly in Asia (61%) and African Region (26%). China is one of 30 high TB burden countries by incident cases in 2015 just after India, Indonesia (1). In China, TB is a major public health problem with the highest absolute number of cases worldwide. Qinghai province lies on the Qinghai-Tibet Plateau with an average altitude of over 3000 meters, which results in low oxygen and low pressure in the region. This province is highly endemic for MTB, among 5.8 million people in this province, about 1.7 million people infected with TB and most of them were rural patients. Several papers have reported geographically structured populations in human pathogens (2-6). Unique geographical location and harsh natural environment and long-lived inhabitants of Tibetan in Qinghai may probably lead to adaptive genetic changes between hypoxia environment, host and pathogen of *Mycobacteria tuberculosis*. Based on this assumption, we conducted molecular studies among isolates of *Mycobacterium tuberculosis* circulating in Qinghai Plateau by Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) and spoligotyping.

MIRU-VNTR analysis (7-9) is a simple and quick genotyping method with a similar discriminatory power to that of RFLP (10). Thus the optimized 15 to 24-locus MIRU-VNTR typing system has been proposed for international standardization (11). Spoligotyping is a rapid and reliable method which based on DNA polymorphisms within the direct repeat (DR) locus of *M. tuberculosis* complex and is well suited for the identification of Beijing family *M. tuberculosis* strains (12). The sequencing and comparison of the genomes of *M. tuberculosis* H37Rv and *Mycobacterium bovis* has revealed large sequence polymorphisms (LSPs), which allowed the recognition of main phylogeographical lineages, resulting from the evolution of the *M. tuberculosis* complex members in association to their host populations (13-15) and RD105 was found to be specific to W-Beijing isolates (16).

Although molecular papers on *M. tuberculosis* have been carried out in China, no genetic diversity study was reported in Qinghai-Tibet plateau. In this study, spoligotyping and 15-locus MIRU-VNTR typing methods were applied for fingerprinting among 251 MTB isolates collected in Qinghai to better understand molecular characteristics and genetic diversities of MTB in this region.

## MATERIALS AND METHODS

### Clinical isolates of *M. tuberculosis*

A total of 251 clinical isolates of *M. tuberculosis* were from the Qinghai Center for Disease Control and Prevention. H37Rv from Chinese Center for Disease Control and Prevention (CDC) were used as reference control.

### Culture, identification and genomic DNA extraction

All clinical isolates cultured in conventional Lowenstein-Jensen (L-J) media at 37°C for 6 to 8 weeks and were identified by means of standard microbiological and biochemical methods (17). Colonies of *M. tuberculosis* was collected from medium using the sterilized saline water and inactivated at 80 for 30 minutes. Precipitated bacteria by centrifugation for 3 minutes at 12000 rpm and then suspended with sterilized saline water and heated in water at 100°C for 1 hour. Supernatants containing the genomic DNA were collected by centrifugation for 3 minutes at 12000 rpm.

### Genotyping methods

#### (1) Spoligotyping

Spoligotyping was performed by amplifying the whole Direct Repeat (DR) region basically as described by Kamerbeek et al. (18). The amplified PCR products were hybridized with nitrocellulose membrane having covalently linked 43 spacer oligonucleotides following the manufacturer's instructions. The results of spoligotyping were compared in binary format with the SpolDB4 database (<http://www.pasteur-guadeloupe.fr/tb/spolddb4>).

#### (2) VNTR typing

VNTR typing was performed by PCR amplification of 15 loci (MIRU10,

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Received: October 25, 2017, Accepted: January 10, 2018, Published: January 19, 2018



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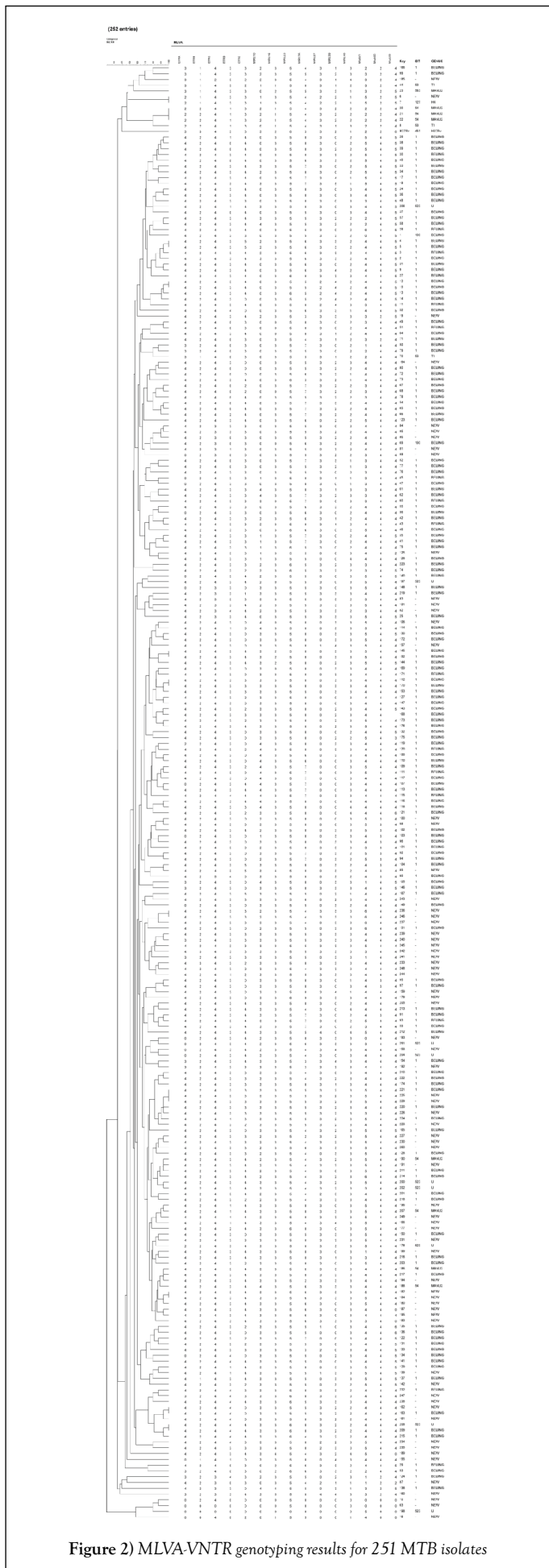


Figure 2) MLVA-VNTR genotyping results for 251 MTB isolates

Vietnam have high proportion of Beijing/W isolates (23-25). In this study, we assessed the prevalence of *M. tuberculosis* Beijing genotype by spoligotyping and MIRU-VNTR.

Previous studies reported that Beijing family strains in northern China was higher than that in the southern region and might relate to geographical location, climate, population composition and other factors (26). Spoligotyping of 251 isolates were grouped 185 (74.0%) into Beijing family and 66 into non-Beijing family, the former was significantly higher than the later. This suggests that the Beijing genotypes are dominant in Qinghai, which is similar to reported papers (19,27). 251 isolates revealed 49 spoligotyping profiles and distributed 217 isolates into 15 clusters with the clustering rate of 86.5% (217/251) (Figure 2). 34 isolates were not clustered. We observed a low diversity (*D*) among tested isolates which was calculated by dividing the number of different patterns by the number of isolates analyzed (28). The estimated degree of diversity was 19.5 ( $D=49/251 \times 100$ ) by spoligotyping. 61 isolates with novel spoligotyping patterns need to be further studied in the future.

VNTR loci were reported could be good markers for phylogenetic estimation (29). In this study, we distributed 251 isolates into 4 groups containing 238 different genetic patterns by 15-locus VNTR analysis (Figure 2). The estimated degree of diversity was 94.8 ( $D=238/251 \times 100$ ) which was much higher than that of spoligotyping, and the clustering rate was 85.7%(215/251) which was well matched with 86.5% by spoligotyping. This high genetic diversity of MTB strains by 15- locus VNTR demonstrates that Multiple-Locus Variable number tandem repeat Analysis (MLVA) would be useful for tracing transmission source.

Group II was found be the largest group in Qinghai province with 91.9 %(230/251) isolates, which was different from Ningbo of China, where the Group III was the main group with 65.6% of tested isolates (30). The founding suggests us more research need to do on pathogenicity, virulence and drug resistance among Group II MTB isolates which would be helpful in better control of MTB in this geographic region.

In conclusion, this is the first study to explore the molecular diversity of *M. tuberculosis* in Qinghai plateau by different typing methods. Our findings in current study may shed light on understanding molecular epidemiology and may allow the development of better strategies for TB control in this region.

TABLE 1  
HGI index of VNTR-Loci for 251 *M. tuberculosis* Clinical trains

VNTR loci	All strains HGI	Beijing family HGI
ETRA	0.316	0.24
ETRB	0.122	0.07
ETRC	0.149	0.07
ETRD	0.436	0.39
ETRE	0.716	0.73
MIRU10	0.686	0.69
MIRU16	0.215	0.15
MIRU23	0.127	0.12
MIRU26	0.768	0.76
MIRU27	0.495	0.52
MIRU39	0.728	0.74
MIRU40	0.647	0.65
Mtub21	0.677	0.65
Mtub30	0.259	0.18
Mtub39	0.435	0.44

TABLE 2  
Comparison of spoligotyping and MLVA-VNTR [n(%)]

Family	Spoligotyping	15 VNTR-locis
Beijing	156 (62.1)	156 (62.1)
like-Beijing	29 (11.6)	29 (11.6)
U	9 (3.6)	9 (3.6)
MANU2	8 (3.2)	8 (3.2)
T1	3 (1.2)	3 (1.2)
H4	1 (0.4)	1 (0.4)
New	45 (17.9)	45 (17.9)

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**FUNDING**

This work was supported by grant from the National Natural Science Foundation of China (No.81160356 and No.81660556)

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**CONFLICT OF INTEREST**

The authors of this paper declare no conflict of interest.

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**ACKNOWLEDGEMENTS**

We thank all the people who made their contributions in collecting samples and conducting phenotypic tests for identification.

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